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Polycomb Group (PcG) Proteins and Human Cancers: Multifaceted Functions and Therapeutic Implications

Wei Wang^{1,2}, Jiang-Jiang Qin¹, Sukesh Voruganti¹, Subhasree Nag¹, Jianwei Zhou³, and
Ruiwen Zhang^{1,2,*}

¹Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health
Sciences Center, Amarillo, Texas, 79106 USA

²Center for Cancer Biology and Therapy, School of Pharmacy, Texas Tech University Health
Sciences Center, Amarillo, Texas, 79106 USA

³Department of Molecular Cell Biology and Toxicology, Cancer Center, School of Public Health,
Nanjing Medical University, Nanjing, P. R. China

Abstract

Polycomb group (PcG) proteins are transcriptional repressors that regulate several crucial developmental and physiological processes in the cell. More recently, they have been found to play important roles in human carcinogenesis and cancer development and progression. The deregulation and dysfunction of PcG proteins often lead to blocking or inappropriate activation of developmental pathways, enhancing cellular proliferation, inhibiting apoptosis, and increasing the cancer stem cell population. Genetic and molecular investigations of PcG proteins have long been focused on their PcG functions. However, PcG proteins have recently been shown to exert non-polycomb functions, contributing to the regulation of diverse cellular functions. We and others have demonstrated that PcG proteins regulate the expression and function of several oncogenes and tumor suppressor genes in a PcG-independent manner, and PcG proteins are associated with the survival of patients with cancer. In this review, we summarize the recent advances in the research on PcG proteins, including both the polycomb-repressive and non-polycomb functions. We specifically focus on the mechanisms by which PcG proteins play roles in cancer initiation, development, and progression. Finally, we discuss the potential value of PcG proteins as molecular biomarkers for the diagnosis and prognosis of cancer, and as molecular targets for cancer therapy.

Keywords

polycomb group proteins; polycomb repressive complex; cancer; oncogene; tumor suppressor

*Correspondence to: Dr. Ruiwen Zhang, M.D., Ph.D., D.A.B.T., Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, 1300 S. Coulter St., Amarillo, TX 79106, USA. T: 806-414-9248; F: 806-356-4034; ruiwen.zhang@ttuhsc.edu.

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1. Introduction

Polycomb group (PcG) proteins were first discovered as epigenetic transcriptional repressors of homeotic gene (*Hox*) expression in *Drosophila melanogaster*, which allows them to control the anterior-posterior segment identity, differentiation, and body planning.¹⁻⁴ Since then, PcG proteins have been identified in all metazoans, exhibiting a remarkable degree of evolutionary conservation from *Drosophila* to humans.^{5,6} PcG proteins have been shown to regulate diverse biological processes during embryonic development, such as cell fate and lineage decisions, cellular memory, stem cell function, and tissue homeostasis.⁷⁻¹³ PcG targets include diverse genes encoding transcription factors, receptors, signaling proteins, morphogens, and regulators involved in all major developmental pathways.⁸ During embryonic development, the PcG proteins and other epigenetic regulators participate in regulation of the transcriptional program, in which the primordial pluripotent embryonic stem cells exhibit temporally restricted transcriptional activation and repression of specific genes. Once completed, the regulated transcriptional program would bestow upon the cells a unique identity and function.¹⁴ Additionally, PcG proteins help these adult differentiated cells to maintain their characteristic gene expression patterns, thus mediating cellular fate and memory.¹⁵⁻¹⁷

During embryonic development, PcG proteins selectively repress gene expression via the formation of multi-subunit complexes termed polycomb repressive complexes (PRCs), which regulate chromatin organization and maintain it in a transcriptionally inactive state.¹⁸ The PRCs basically comprise PRC1 and PRC2. Both PRC1 and PRC2 induce covalent post-translational histone modifications.^{19,20} While the PRC1 subunits catalyze the monoubiquitination of histone H2A at lysine 119 (H2AK119Ub1),²¹ the PRC2 subunits catalyze the trimethylation of histone H3 at lysine 27 (H3K27me3).²² Both of these post-translational modifications of histones are associated with transcriptional silencing.^{23,24} In addition, other distinct PcG complexes have been identified, mainly in *Drosophila*, including the pleiohomeotic protein (Pho) repressive complex (PhoRC), dRing-associated factors (dRAF) complex, and Pc-repressive deubiquitinase (PR-DUB) complex.²⁵ dRAF and PR-DUB mostly function as accessory complexes, interacting with PRC1/PRC2 to collectively form robust repressive machinery.^{26,27} Interestingly, PhoRC occupies some targets without PRC1 and PRC2, suggesting other roles independent on PRC complexes.²⁸

There exists a strong link between PcG proteins and cancer; several cancer types exhibit deregulation of the PcG protein expression and function.^{14, 29-34} Since PcG proteins play an important role in determining cellular differentiation and lineage, abnormal PcG expression and/or mutations lead to impaired signaling that inhibits tumor suppressors and falsely activates proto-oncogenes. This leads to a loss of cell identity, and confers persistent proliferative ability, resistance to cell death mechanisms, bypass of cellular senescence programs, and increased migratory/invasive potential.^{12-14,29-34} Several recent reviews have comprehensively discussed PcG-mediated transcriptional repression, including the role of PcG proteins in stem cell renewal and lineage commitment.³⁰⁻³⁴ Recent studies have indicated that PcG proteins may also regulate cellular and oncogenic functions in a transcription repression-independent manner. These functions may be termed the 'non-polycomb functions' of the PcG proteins.

In this review, we compare both the classical-polycomb and non-classical-polycomb functions of PcG proteins to provide a better understanding of their significance in cancer development and progression. We also focus on the myriad roles of PcG proteins in diverse cellular functions, focusing on oncogenesis, as well as their utility as prognostic or therapeutic markers of human cancers. Finally, we discuss the strategies for targeting the PcG proteins in cancers, and critically evaluate their validity as viable therapeutic targets.

2. PcG-Mediated Transcriptional Repression

PcG proteins act as silencers of global gene expression, most importantly regulating normal embryonic development and differentiation in all metazoans, as illustrated in Figure 1. PcG proteins are required for the correct spatial expression of homeotic (*Hox*) genes during development. In *Drosophila*, PcG mutants are characterized by extreme adult morphologies due to inappropriate activation of *Hox* genes.⁴ PcG proteins are also implicated in diverse genetic and cellular processes, including X-inactivation,³⁵ cell cycle progression,³⁶ senescence,³⁷ cell fate decisions,¹⁴ and stem cell differentiation.³⁰ Of particular importance is the role played by the PcG proteins in tumorigenesis.^{12-14,29-34} As discussed previously, PcG proteins control gene expression via mediating changes in chromatin structure and function that regulate the accessibility of genetic material to regulatory proteins.³⁹ Heterochromatization by PRC2 (involving the local methylation of histone H3 on either lysine 9 (H3K9me3) or lysine 27 (H3K27me3)) is a key signature in several cancer types, especially prostate and colon cancers.⁴⁰⁻⁴² These repressive chromatin marks contribute to cancer-associated *de novo* DNA methylation and gene silencing that play a role in normal cellular differentiation and function (Figure 1), such as cell proliferation inhibitors, cell adhesion promoters, *etc.*^{43,44}

A. Polycomb Repressive Complexes (PRCs)

As discussed previously, PcG proteins function principally as two large multisubunit complexes, PRC1 and PRC2. Although the exact composition of these complexes varies based on the cell type and function, their core components are conserved.⁴⁵ As shown in Figure 2, PRC1 consists of polycomb group ring finger proteins [PCGF, posterior sex combs (Psc) in *Drosophila*], really interesting new gene 1/2 [RING1/2, sex combs extra (Sce)/RING in *Drosophila*], chromobox (CBX, polycomb (Pc) in *Drosophila*), and polyhomeotic-like protein (PHC, Ph in *Drosophila*).^{46,47} PRC1 is formed around the RING1/2 subunits, to which is bound one of the six alternative PCGF1-6 proteins, such as moloney murine leukemia virus integration site 1 (BMI1, also known as PCGF4) or MEL18 (also known as PCGF2). The RING-PCGF heterodimers constitute the canonical PRC1 variants and possess characteristic PHC and CBX subunits, which have the ability to recognize the H3K27me3 mark via the chromodomain of the CBX subunit.^{48,49} On the other hand, the RING-PCGF heterodimers also form non-canonical PRC1 complexes, containing RING1 and Ying Yang 1 (YY1) binding protein (RYBP) instead of PHC and CBX, which have a much higher capacity for ubiquitinating H2A.⁵¹ As shown in Figure 2, the variant non-canonical complexes also possess different additional subunits.

Similarly, PRC2 essentially consists of enhancer of zeste homolog 2 [EZH2, E(Z) in *Drosophila*], ectodermic embryonic development [EED, extra sex combs (Esc) in

Drosophila], suppressor of zeste 12 [SUZ12, Su(z)12 in *Drosophila*] and retinoblastoma-associated protein 46 [RbAp46, nucleosome-remodeling factor 55 kDa subunit (Nurf55) in *Drosophila*].^{51,52} In mammalian systems, EZH2 acts as the catalytic subunit of this complex but requires the other factors for activation.⁵³ SUZ12 also critically regulates the histone methyltransferase (HMT) activity of PRC2,⁵⁴ while EED modulates the substrate specificity of the EZH2 complex, resulting in differential targeting of its HMT activity toward histone H3K27 or histone H1K26 (Figure 2).⁵⁵ EZH2 primarily catalyzes H3K27me3, a mark of transcriptionally repressive chromatin and mediates transcriptional silencing.⁵¹ EZH1, a homolog of EZH2, partially complements EZH2 in mediating H3K27me3 and safeguarding stem cell identity.⁵⁶ Margueron *et al.* report that the EZH1 and EZH2 exhibit contrasting roles in repression.⁵⁸ EZH2 knockdown markedly decreases global H3K27me2/3 levels, and EZH1 knockdown shows no effect on histone methylation. Interestingly, EZH1-mediated repression is independently of histone methylation and EZH1 directly compacts chromatin in the absence of S-adenosyl-L-methionine (SAM), a methyltransferase cofactor.⁵⁷ During myogenic differentiation, PRC2-EZH2 binds to myogenin (MyoG) promoter and muscle creatine kinase (mCK) enhancer in myoblasts and PRC2-EZH1 replaces PRC2-EZH2 on MyoG promoter in myotubes, and these two chromatin dynamics are regulated by different mechanism.⁵⁸ PRC2 also has a role in X chromosome inactivation, in the maintenance of stem cell fate, and in imprinting.¹⁹

A third PcG complex involved in homeotic gene silencing, PhoRC, has been identified. PhoRC contains the sequence-specific DNA-binding transcription factor, YY1 (Pho in *Drosophila*),⁵⁹ as well as a scm-like with four MBT domains protein 1 (Sfmbt1, dSfmbt in *Drosophila*),⁶⁰ which specifically binds to mono- and di-methylated K3K9 and H4K20 peptides. No enzymatic activity has been shown to be associated with PhoRC, suggesting that PhoRC may direct PRC1 or PRC2.^{59,60} Of note, the Pho complex activity is controversial in the field. An overrepresented motifs analysis in human core promoters demonstrates YY1 plays a dual regulatory role in transcription and initiation of translation, but ChIP sequencing results does not highlighted an overlap with PRC1 and PRC2.⁶¹ The functions of other reported polycomb complexes, such as dRAF and PR-DUB, have not yet been deciphered in mammals or other higher organisms.^{27,62} An overview of various PRC subunits and their known biological functions is presented in Table I.

B. Regulation of Gene Expression by PRCs

The PRCs have specific catalytic functions that regulate their ability to repress transcription and their own biological activity. The recruitment of the PRCs to specific targets is generally thought to occur in two steps: first, PRC2 produces the H3K27me3 mark at a specific gene, then the PRC1 complexes are recruited by their ability to recognize and bind to H3K27me3.¹⁰⁶ However, recent studies indicate that the binding of a variant PRC1 complex (containing lysine (K)-specific demethylase 2B (KDM2B)) and the subsequent H2A ubiquitination of surrounding chromatin are sufficient to elicit PRC2 recruitment and H3K27 trimethylation.¹⁰⁷⁻¹⁰⁹ These studies indicate that PRC2 can directly recognize ubiquitinated H2A, and the disruption of PRC1-mediated H2A ubiquitination impairs genome-wide PRC2 binding.¹⁰⁹ As a result of the RING1/2 activity, nucleosomes adjoining the binding site become ubiquitinated,²⁶ along with the canonical PRC1 subunits.¹⁰⁷ PRC1

also modifies chromatin by a non-covalent mechanism; it inhibits nucleosome remodeling by SWItch/sucrose nonfermentable (SWI/SNF) complexes and compacts nucleosome arrays *in vitro*.¹¹⁰ PRC2 exhibits methyltransferase activity towards lysine 26 of the linker histone H1, which can then bind heterochromatin binding protein 1 (HP1) to chromatin, promoting chromatin compaction.¹¹¹ Recent studies also indicate that the PRC2 subunit EZH2 can recruit DNA methyltransferases (DNMTs) to select target genes, leading to DNA hypermethylation and subsequent transcriptional repression.¹¹²

C. PcG Recruitment to Target Genes

PcG proteins regulate gene transcription in a cell-type-specific manner, *i.e.*, binding to different sets of genes in different cell types. The mechanisms and DNA elements regulating the binding and dissociation of PcG proteins to their target promoters are thought to be highly specific. Since PcG proteins do not possess any known DNA binding activity, PcG-mediated target genes repression requires specific cis-regulatory sequences, called polycomb response elements (PREs), for recruitment to and displacement from their target genes (Figures 3A and 3B). PREs have been characterized in *Drosophila* where they correspond to a specific DNA consensus.¹¹³ In fact, most PcG proteins are seen to be specifically bound at the PREs of target genes.¹⁹ PcG protein recruitment depends on the combined actions of several sequence-specific DNA-binding proteins, such as Pho and its homolog, pleiohomeotic-like (PhoI), as well as dorsal switch protein 1 (Dsp1), zeste, grainy head (Grh), GAGA factor (GAF; Trithorax-like), and pipsqueak (Psq).^{45,114} These DNA binding proteins recognize several conserved sequence motifs at or near PREs, leading to the binding of PcG proteins to their targets.^{45,114}

On the other hand, in mammals, the recruitment is much more complicated and few sequences with PRE features have been identified, and a conserved consensus has not been found.¹¹⁵ The candidate central recruiter proteins, CpG islands, HIGH MOBILITY GROUP BOX 2 (HMGB2, the mammalian ortholog of Dsp1), and YY1 are involved in PcG recruitment.¹¹⁶ For example, HMGB2 and YY1 forms a complex to mediate transcriptional repression.¹¹⁷ EZH2 is recruited to muscle-specific genes by YY1, where it prevents the differentiation of myoblasts.¹¹⁶ Apart from specific transcription factors that recruit PcG proteins to PREs, long non-coding RNAs (ncRNAs) have also been shown to recruit PcG proteins.¹¹⁸ Long ncRNAs are able to recruit both PRC1 and PRC2 to chromatin through interactions with the chromodomains and SET in PRC1 and PRC2, respectively.¹¹⁸⁻¹²⁰ These ncRNAs have been suggested to bind specifically to the promoters of their target genomic sequences and recruit polycomb complexes to interact with PRC2.¹²⁰ In certain cases, accessory proteins, such as ATP-dependent helicase ATRX (also known as X-linked helicase II), directly interact with RepA/Xist ncRNA to promote the loading of PRC2 *in vivo*.¹²¹

D. Regulation of PRCs

The transcriptional activity of PcG protein complexes is modulated at various levels. These include a balance between the active and inactive chromatin marks, diverse post-translational modifications of the PRC subunits, and interactions with other signaling molecules. The presence of active chromatin marks (*e.g.*, H3K4me3, H3K36me2, or

H3K36me3) attenuates the catalytic activity of PRC2, while nucleosome compaction and the presence of H3K27me2 or H3K27me3 stimulate its catalytic activity, thus favoring its role in the maintenance of transcriptional state.^{122,123}

The various PRC1 and PRC2 subunits are also regulated by post-translational modifications.³³ Extracellular signals induce Akt-mediated phosphorylation of EZH2 at Serine 21 (Ser21), which results in a suppression of the PRC2 activity.^{124,125} Akt-dependent phosphorylation does not affect EZH2 binding with other PRC2 components, but does reduce the affinity of EZH2 for histone H3, leading to H3K27me3 and consequent de-repression of the EZH2-silenced genes.¹²⁵ BMI1 also undergoes phosphorylation at Ser316 by Akt, and phosphorylated BMI1 is dissociated from the inhibitor of cyclin-dependent kinase 4-alternative reading frame (INK4a-ARF) locus, which results in decreased histone H2A ubiquitination and decreased tumor growth and self-renewal of hematopoietic stem and progenitor cells.¹²⁶ Another PRC2 subunit, SUZ12, also undergoes sumoylation both *in vitro* and *in vivo*.¹²⁷ Interestingly, a PRC1 subunit, CBX4, acts as an E3 small ubiquitin-related modifier (SUMO) transferase and mediates the sumoylation of BMI1 and the subsequent recruitment of PRC1 to sites of DNA damage.¹²⁸ The phosphorylation of various PRC1 components has been reported (*e.g.*, Akt and mitogen-activated protein kinases (MAPK)-mediated BMI1 phosphorylation),¹²⁹ but their functions in regulating polycomb-mediated gene repression are not well understood.

Several other proteins interact with PRC subunits and regulate their activity. B-cell lymphoma 6 (BCL6) interacting co-repressor (BCOR) forms a complex with RING1/2, RYBP, PCGF1, and KDM2B, which is then recruited to BCL6 target genes, causing their transcriptional repression.¹³⁰ Human plant homeodomain (PHD) finger protein 1 [PHF1, polycomb-like (PCL) in *drosophila*], also interacts with PRC2 and modulates PRC2's HMT activity, but its effect on the H3K27me3 levels is unclear.¹³¹ In acute promyelocytic leukemia (APL), the oncogenic transcription factor, promyelocytic leukemia-retinoic acid receptor alpha (PML-RAR α), recruits PRC2, the nucleosome remodeling and deacetylation (NuRD) complex, and DNMT to the retinoic acid receptor β 2 (RAR β 2) promoter in a precise sequence of events.^{132,133} The pro-invasion molecule snail homolog 1 (SNAIL1) (mammals) also interacts with PRC2 and recruits it to the E-cadherin promoter, causing its transcriptional repression and promoting cell invasion and the epithelial–mesenchymal transition (EMT).¹³⁴ The sonic hedgehog (Shh) ligand induces BMI1 expression, and BMI1 is overexpressed in medulloblastomas, where the Shh pathway is constitutively activated.¹³⁵

E. Cell Fate Determination and Cellular Differentiation Induced by PRCs

PcG proteins regulate cell fate decisions during embryonic development and cellular differentiation via the selective activation of particular sets of genes and repression of others via dynamic switches in PcG-associated histone marks and DNA methylation.¹³⁶ Prior to differentiation, a cell expresses genes characteristic of pluripotency (the so-called ‘stem cell genes’) that maintain it in a proliferative undifferentiated state. During this state, the PcG proteins repress the transcription of the genes involved in lineage-specific differentiation. As signals promoting cellular differentiation are activated, PcG proteins are recruited to these ‘genes’, where they silence their expression, thereby ‘locking’ cell fate and maintaining the

cellular identity through subsequent cell divisions.¹³⁷ PcG complexes also bind to and repress numerous genes that encode key developmental regulators and signaling proteins in embryonic stem cells.^{33,138} The PcG proteins and chromatin interaction is associated with increased levels of H3K27me3 and H2AK119Ub1.¹³⁹ Knockdown of the PRC2 and PRC1 genes results in a spontaneous increase in developmental gene expression, and consequently, in differentiation defects of both stem cells as well as somatic tissues.³³ Moreover, inappropriate expression of these genes in immature cells leads to premature lineage specification associated with a loss of stem and progenitor cell populations.¹⁴⁰

3. Non Classical-Polycomb Functions of PcG Proteins

In the previous sections, we have discussed the polycomb functions of PcG proteins, *i.e.* transcriptional repression *via* reordering of the chromatin structure and/or the generation of repressive chromatin marks. Recent studies have demonstrated that PcG proteins may interact with other signaling pathways, independent of the aforementioned polycomb functions. It is now thought, at least in certain scenarios, that PcG activity does not depend on global gene silencing. In this section, we present evidence of such non classical-polycomb functions.

A. Target Genes

EZH2 silences gene transcription by generating the H3K27me3 repressive chromatin mark *via* its SET domain,¹⁴¹ and is frequently overexpressed in many cancer types, including breast, lung, and prostate cancer.^{142,143} In castration-resistant prostate cancer (CRPC), EZH2 has been shown to repress two tumor suppressor genes in prostate cancer, adrenoceptor beta 2 (ADRB2)¹⁴⁵ and DAB2 interacting protein (DAB2IP)¹⁴⁶. EZH2 also represses tumor suppressor genes beta-microseminoprotein (MSMB), which encodes prostatic secretory protein 94 amino acids (PSP94)¹⁴⁷ and human DOC-2/DAB2 interactive protein (hDAB2IP, also known as ASK-interacting protein 1 (AIP1))¹⁴⁶. While MSMB promoter binds to PRC2 and H3K27me3, hDAB2IP promoter is occupied by PRC2 and EZH2. In breast cancer, the tumor suppressor E-cadherin is identified as one of key targets of EZH2 by cDNA microarray analyses and ChIP-on-chip measurements. EZH2 mediates repression of E-cadherin transcript by H3K27me3 activation. The promoter of E-cadherin is bound to PRC2 complex when EZH2 is overexpressed and this effect is blocked by Histone deacetylase (HDAC) inhibitors.¹⁴⁸ EZH2 also represses DNA repairs associated genes RAD51 paralog mRNA levels. The RAD51 paralogs are required for hampered double-strand break (DSB) repair, which lead to chromosomal abnormalities and result in cancer.¹⁴⁹ Interestingly, in an ER-positive environment, ectopically overexpressed EZH2 interacts with the Wnt signaling components, T cell factor (TCF) and β -catenin, as well as with the ER, and leads to activation of the c-Myc and Cyclin D1 genes,¹⁵⁰ independent of its methyltransferase activity (Figure 4A).

Chromobox homolog 7 (CBX7), which is earlier thought to have few roles beyond its function as a member of the PRC1 family, has been shown to physically interact with HDAC2 and inhibit its activity on the E-cadherin promoter, thus increasing its expression levels.¹⁵¹ CBX7 has also been shown to increase E-cadherin levels by a direct interaction with the E-cadherin promoter.¹⁵¹ The expression of CBX7 increases the acetylation status of

histones H3 and H4 on the E-cadherin promoter. Not surprisingly, the loss of CBX7 leads to highly malignant phenotypes of thyroid cancers.¹⁵²

B. Target Proteins

In estrogen receptor (ER)-negative breast cancer, EZH2 drives cancer progression *via* its physical interaction with the v-rel avian reticuloendotheliosis viral oncogene homolog A (RelA) subunit of nuclear factor-kappaB (NFκB), independent of its histone methyltransferase activity (Figure 4B).¹⁵³ In CRPC, the oncogenic role of EZH2 is based on the transcriptional induction of a subset of its target genes (Figure 4C).¹⁵⁴ EZH2 phosphorylation acts as a co-activator for several critical transcription factors, such as the androgen receptor (AR), leading to advanced castration-resistant forms of the disease.¹⁵⁴ Although EZH2, but not other PRC2 subunits, is overexpressed in androgen-independent LNCaP (abl) cells, it is found that the EZH2-associated H3K27me3 is lower than that in the parental androgen-sensitive LNCaP cells. Upon investigation of the genome-wide location of EZH2, it is discovered that a subset of EZH2-bound genes do not bind the PRC2 subunit, SUZ12, or exhibit H3K27me3.¹⁵⁴ Many of these target genes are down-regulated upon EZH2 knockdown, implicating a PRC2-independent role for EZH2 in their activation. Furthermore, EZH2 depletion does not change the AR levels, but decreases AR-associated lysine methylation, leading to a change in the induction of its target genes. Apparently, the interaction of EZH2 with the AR is mediated by the phosphorylation of EZH2 at Ser21 by Akt, which decreases its interaction with H3K27.¹⁵⁴ In hepatocellular carcinoma (HCC), EZH2 has been shown to regulate p53 expression in an H3K27me3-independent manner.¹⁵⁵ As discussed previously, the trimethylation mark on H3K27 is a characteristic repressive mark of PRC2, *via* which it mediates its transcriptional silencing activities. Thus, H3K27me3-independent p53 down-regulation by EZH2 in HCC is a polycomb-independent function of EZH2.

The PRC1 subunit, BMI1, is deregulated in several cancer types and promotes the EMT *via* E-cadherin down-regulation in association with the pro-invasive SNAIL protein.¹³⁴ These activities require H3K27me3 repressive marks on the target genes, and are indicative of traditional polycomb activity. BMI1 activates the WNT pathway by repressing the Dickkopf (DKK) family of WNT inhibitors, leading to the up-regulation of c-Myc, which in turn leads to the transcriptional autoactivation of BMI1 (Figure 5A). This positive feedback loop regulates BMI1's role in malignant transformation and maintenance of the stem cell phenotype.¹⁵⁵ However, through the induction of IκB phosphorylation,¹⁵⁶ BMI1 regulates NFκB activity and promotes NFκB nuclear translocation and the expression of its downstream target genes, such as Bcl-X(L) and c-Myc.¹⁵⁷ Inhibition of the IκB kinase (IKK)-NFκB pathway abolishes the anti-apoptotic effects of BMI1 on glioma cells. In *in vitro* and *in vivo* glioma models, BMI1 upregulated both the NFκB activity and vascular endothelial growth factor C (VEGF-C) expression, promoting angiogenesis and invasion.¹⁵⁸ BMI1 has also been shown to autoactivate its own transcription, which is dependent on the E-box of its promoter, to which c-Myc binds (Figure 5B).¹⁵⁵

RYBP, which belongs to the non-canonical PRC1 family, and acts as a mediator to bridge the PRC1 complex to several sequence-specific transcription factors, such as YY1, GA-

binding protein subunit beta-1 (GABPB1) and E2F transcription factor 6 (E2F6), and represses gene transcription.¹⁵⁹ Our group first demonstrated that RYBP directly binds to the murine double minute 2 (MDM2) oncoprotein, and stabilizes both MDM2 and p53 expression. In fact, RYBP mediated MDM2 stabilization is independent of the p53 status of the cells. The RYBP-MDM2 interaction prevents MDM2-mediated degradation of p53 tumor suppressor, and activates p53 transcriptional activity (Figure 6).⁸⁵ Our recent studies have further indicated that RYBP induces cell death in HCC, independent of p53.¹⁶⁰ In addition, RYBP binds to several apoptotic mediators, including death domains, and enhances apoptosis via the formation of death-inducing signal complexes (Figure 6).^{86,161}

Another PRC1 core subunit, ring finger protein 2 (RNF2, also known as RING2), has been identified as an E3 ligase that targets p53 for degradation.^{162,163} The E3 ligase activity of RNF2 had already been shown to require BMI1.¹⁶⁴ Recently, RNF2 is found to directly bind to both p53 and MDM2, and promotes MDM2-mediated p53 ubiquitination (Figure 6).¹⁶³ RNF2 overexpression also increases the half-life of MDM2 and inhibits its auto-ubiquitination.¹⁶³ PHF1, a PRC2 family member, activates p53 by increasing the p53 protein levels, as well as increasing the stability of the p53 protein.¹⁶⁵ Studies have indicated that PHF1 directly interacts with the p53 protein and co-localizes with p53 in the nucleus *in vitro* and *in vivo*.¹⁶⁶ Mechanistically, PHF1 protects p53 protein from MDM2-mediated ubiquitination and degradation. PHF1 is also involved in DNA double-strand breaks (DSBs) damage response in human cells, promoting nonhomologous end-joining processes through its direct physical interaction with Ku70/Ku80.¹⁶⁶

In melanoma cells, PHF19 regulates the switch between proliferative and invasive phenotypes via modulation of cyclin expression.¹⁶⁷ Of note, the expression of PHF19 itself is regulated by phosphorylated Akt. Whether the regulation occurs via a post-translational modification of PHF19 is currently unknown.

4. PcG Proteins and Cancer

It has been suggested that the sequential acquisition of mutations drives tumorigenesis via the activation of oncogenes and the loss of function of tumor suppressor genes.¹⁶⁸ However, epigenetic changes, including aberrant DNA methylation patterns and post-translational modifications of histones, also play a crucial role in cancer development and progression.¹⁶⁹ Both DNA methylation and histone modification regulate gene expression by influencing the chromatin structure and the accessibility of DNA. An abnormal chromatin structure can cause inappropriate gene expression and genomic instability, resulting in cellular transformation and malignant growth. Therefore, proteins that regulate the chromatin structure constitute key players in the pathogenesis of cancer. As shown in Figure 7, the PcG proteins are an important class of such epigenetic regulators. Several PcG proteins are differentially expressed in malignant tissue compared to corresponding normal tissue, and these have been summarized in Table II.

A. PcG Deregulation in Human Cancer: Clinical Prospective

The deregulation of PcG proteins has been frequently observed in various cancer types. BMI1 is amplified and overexpressed in human leukemia and solid tumors.¹⁷⁵⁻¹⁷⁸ EZH2,

SUZ12, and PCL3/PHF19 are also overexpressed in a variety of human cancers (Table II). The *Ezh2* gene is amplified in several human prostate cancer cell lines, with more than half of the hormone-refractory prostate cancers exhibiting *Ezh2* amplification.¹⁹⁷ In addition to abnormal levels of PcG proteins, human cancers also exhibit several PcG missense mutations and chromosomal translocations.^{174,181-185,189,193} Somatic *Ezh2* mutations and deletions that inactivate the methyltransferase activity of EZH2 are found in B cell lymphomas, follicular lymphomas, and myelodysplastic and myeloproliferative disorders.¹⁹⁸ On the other hand, heterozygous null mutations of *Eed* in mice increase the incidence and reduce the latency of lymphoid tumors, indicating a possible tumor suppressive role for this PRC2 subunit.^{199,200} Heterozygosity for a *Suz12* allele carrying an inactivating point mutation has been reported to cause an increase in the numbers of platelets, megakaryocytes, and hematopoietic progenitors in mice.²⁰¹ Moreover, hematopoietic stem cells (HSCs) harboring a *Suz12* mutation exhibit increased proliferative potential, indicating that SUZ12 negatively regulates the HSC/progenitor activity, and suggesting that SUZ12 has a tumor suppressive role.²⁰¹ Some PcG genes are also involved in some of the recurrent chromosomal rearrangements detected in human cancers (Table II). In addition, PcG proteins physically interact with a number of chimeric fusion proteins involved in leukemia, such as promyelocytic leukemia zinc finger-retinoic acid receptor α (PLZF-RAR α), and induce the transcriptional silencing of target genes.²⁰²

B. PcG Proteins in Cancer Development and Progression: Mechanisms of Action

The first association between the PcG family and cancer development is suggested by the identification and functional characterization of murine BMI1 as a proto-oncogene that promotes B- and T-cell lymphomas via its cooperation with c-Myc.²⁰³ BMI1 inhibits Myc-induced apoptosis through repression of the cyclin-dependent kinase inhibitor 2A (Cdkn2a) locus that encodes two structurally distinct proteins, INK4 and ARF, both of which restrict cellular proliferation in response to aberrant mitogenic signaling.²⁰⁴ INK4 is a cyclin-dependent kinase inhibitor (CDKI) that activates the retinoblastoma (RB) pathway, while ARF induces p53 by inhibiting MDM2 activity.²⁰⁵ Subsequently, several other PcG members, such as EZH2 and SUZ12, have also been linked to tumorigenesis (Table II). The role of PcG proteins in carcinogenesis is thought to primarily involve a bypass of the cellular senescence program due to the transcriptional repression of the INK4b-ARF-INK4a locus.²⁰⁴

As discussed earlier, PcG proteins maintain a chromatin-based embryonic stem cell state and are crucial during embryogenesis. Abnormally elevated levels of PcG proteins may lead to the generation and maintenance of cancer stem cells.^{13,34,38} BMI1 and EZH2 also silence tumor suppressor genes like phosphatase and tensin homolog (PTEN), subsequently activating the phosphoinositide 3-kinase (PI3K)-Akt-glycogen synthase kinase 3 β (GSK3 β) pathway and promoting the EMT and invasion.²⁰⁶⁻²⁰⁸ Representative examples of the various mechanisms by which the PcG proteins regulate cancer development and progression are summarized in Table III.

C. Epigenetic Deregulation of PcG Proteins in Cancer

1. DNA Hypermethylation—DNA hypermethylation leading to the inactivation of tumor suppressor genes is a common feature of many different cancer types.^{221,222} A PcG target methylation signature has been observed in ovarian cancer, follicular lymphoma, and glioblastoma multiforme; several frequently hypermethylated genes in colon or prostate cancers are pre-marked by PcGs and by the PRC2-mediated H3K27me3 mark for *de novo* methylation.²²³ EZH2 interacts with the DNA methyltransferases DNMT1, DNMT3A, and DNMT3B, allowing the recruitment of DNMTs to PRC2-repressed genes and their subsequent methylation.¹¹² CBX4 and CBX7 also interact with DNMT3B, whereas BMI1 forms a ternary complex with DNMT1 through a direct interaction with DNMT-associated protein 1 (DMAP1).²²⁴⁻²²⁶ DNMT3B-PcG-bound genes in normal cells are good predictors of epigenetically-silenced loci in colon cancer.²²⁷ Since PcG proteins are key regulators of cell fate and lineage, gradual *de novo* DNA methylation of PcG targets commits cells to an undifferentiated state and predisposes them to malignant transformation.

2. Noncoding RNAs—Large intervening noncoding RNAs (lincRNA), such as HOTAIR, which recruits the PRC2 complex to silence the *HoxD* cluster, are overexpressed in primary and metastatic breast tumors.^{229,230} Similarly, the lincRNA antisense non-coding RNA in the INK4 locus (ANRIL) and the PRC1 protein CBX7 interact to repress the Ink4b/Ink4a/ARF locus in prostate cancer cells.²³⁰ In recent years, small noncoding RNAs have been recognized as major players in regulatory pathways, and many of them are deregulated in cancers.²³¹ While several miRNAs bind to and inhibit PCG expression (Figure 8A), multiple miRNAs are themselves repressed in embryonic stem cells and are marked by H3K27me3 modification, indicating that PRC2 complexes regulate their expression.²³² For example, it has been demonstrated that H3K27me3-mediated miR-31 silencing leads to the constitutive activation of NFκB in adult T-cell leukemia (Figure 8B).²³³ Furthermore, the fact that miR-31 inhibits breast cancer metastasis has led to speculation about whether miR-31 silencing through PRC2 can occur in other tumor types.²³⁴ In prostate cancer, EZH2 acts as the link between the epigenetic silencing of two pro-apoptotic miRNAs, miR-205 and miR-31. EZH2 represses miR31, while being silenced itself by miR-205. This miR-31 silencing leads to increased expression of the antiapoptotic protein, E2F6. Thus, a decrease in miR-205 expression in prostate cancer correlates with EZH2 overexpression and miR-31 silencing, leading to resistance to chemotherapy-induced apoptosis.²³⁵

In addition, several miRNAs bind PcG transcripts and inhibit their expression, e.g., miR-26a and miR-214 target the *Ezh2* transcript.^{236,237} It is also known that miR-101 targets the 3'UTR of *Ezh2* mRNA and promotes its degradation.^{238,239} Supporting its role in cancer development or progression, miR-101 is down-regulated in bladder transitional cell carcinoma, hepatocellular carcinoma, and prostate tumors, and this correlates with high EZH2 levels.²⁴⁰⁻²⁴² A large proportion of advanced tumors harbors a loss of one or both miR-101 alleles.²⁴² Overexpression of miR-101 inhibits the proliferation and reduces the invasiveness potential and colony formation activity of cancer cells, resulting in a phenotype similar to that found after EZH2 knockdown.²⁴³ BMI1 is also targeted by multiple miRNAs. The expression of BMI1 is post-transcriptionally inhibited by miR-128, miR-183, miR-200c, and miR-203, which target the 3'UTR of the transcript.²⁴⁴⁻²⁴⁷ In invasive cancers, these

miRNAs are often suppressed by DNA methylation, leading to increased levels of the oncogenic PcG proteins like EZH2 and BMI1. The suppression of BMI1-targeting miRNAs, like mir-200c, mir-203, and mir-183, by the pro-invasion Zinc finger E-box-binding homeobox 1 (ZEB1) protein also promote the EMT and maintenance of stem cell self-renewal.^{248,249}

5. PcGS Proteins as Diagnostic and Prognostic Biomarkers for Cancer

In previous sections, we have discussed the critical roles of PcG proteins in cancer initiation, development, and progression. In this section, we discuss the importance of various PcG proteins as diagnostic and/or prognostic markers for human cancers.

A. EZH2

EZH2 is upregulated in a wide range of human cancers, and its expression correlates with tumor aggressiveness across all cancer types tested (Table II).^{153,154,185-187} It is a particularly important factor for assessing the progression and prognosis of breast^{187,250,251} and prostate cancers.^{186,251} Increased expression of EZH2 in normal breast tissue predisposes the subject to eventual cancer development.^{252,253} Abnormal EZH2 expression contributes to genomic instability and drives malignant transformation.²⁵⁴ Similarly, in glioblastomas, EZH2 inhibits differentiation and activates genes that regulate cell proliferation, cell cycle progression, and cell migration.²⁵⁵⁻²⁵⁷

B. BMI1

A high level of BMI1 expression predicts advanced disease and a poor prognosis in several human cancer types (Table II).¹⁷⁵⁻¹⁷⁸ The expression of BMI1 is often associated with aggressive and chemoresistant stem cell-like cancers, and can be indicative of mortality.²⁵⁸⁻²⁶¹ In precancerous tissues and biopsies of cancer, elevated BMI1 expression is found to be predictive of eventual far-site metastases, thus serving as an invaluable diagnostic/prognostic tool.^{262,263} However, a high level of BMI1 expression may not correlate with a poor prognosis for all cancer types. For example, high levels of BMI1 are corresponded to a better overall survival in patients with breast cancer,^{264,265} endometrial carcinomas,²⁶⁶ and malignant melanoma.²⁶⁷ In renal carcinoma, BMI1 expression is found to be inversely correlated with the differentiation grade of the carcinoma, acting as a differentiation marker that is lost in high-grade carcinomas.²⁶⁸ These results indicate that the behavior of BMI1, with respect to cancer progression, is dependent on the cell type and context. It must, therefore, be analyzed with this in mind when developing targeted therapeutic strategies for patients.

It is possible that the inconsistent relationship between BMI1 expression and the prognosis is related to its role in DNA repair.^{269,270} Gieni and Hendzel speculate that in cancers where genomic instability is crucial to the pathology, the absence of BMI1 expression increases the accumulation of genetic damage, perhaps leading to the less favorable prognosis noted in these studies.¹³ This hypothesis agrees well with the fact that aggressive breast cancer subtypes are accompanied by low tumor expression of BMI1 and mutations in other proteins involved in DNA repair, such as breast cancer genes 1/2 (BRCA1/2).^{13,271}

C. Other Proteins

The connection between other PcG proteins and cancer is less clear. SUZ12 is upregulated or anomalously expressed in human colon,¹⁹⁰ breast,¹⁹⁰ and lung tumors,²⁷² while RING1 is overexpressed, along with BMI1 and EZH2, in prostate cancer.²⁷³ However, their expression levels do not have independent prognostic value.²⁷³ In contrast, low levels of *CBX7* mRNA correlate with disease progression and a negative prognosis in bladder carcinoma,²⁷⁴ while the loss of CBX7 protein expression correlates with a highly malignant phenotype in thyroid cancer,¹⁵² indicating that CBX7 regulates genes involved in cancer progression. Low levels of CBX8 were associated with a poor prognosis and high rate of distant metastasis in colorectal cancer patients.²⁷⁵ MEL18, the PRC1 subunit, exhibits tumor suppressor activity through inhibition of BMI1 expression.²⁷⁶ However, the tumor suppressor role of MEL18 is challenged by evidence that BMI1 and MEL18 have overlapping functions in the growth of human medulloblastoma cancer cells.²⁷⁷ We have recently demonstrated that RYBP is down-regulated in HCC, with its loss contributing to a poor prognosis and decreased chemosensitivity.¹⁶⁰ An increased response to chemotherapeutic drugs has been achieved by adenoviral delivery of RYBP into test subjects, further supporting its role in the response to chemotherapy.^{160,278}

6. Targeting PcG Proteins for Cancer Prevention and Therapy

Our discussion, so far, underscores the key roles played by the PcG proteins in the regulation of cancer development and progression. Since PcG proteins such as EZH2 and BMI1 have been proven to be *bona fide* oncogenes and cancer stem cell markers, PcG proteins appear to be attractive targets for both cancer prevention and therapy. We will discuss the validity of PcG proteins as preventive and therapeutic targets below (Figure 9).

A. Cancer Prevention

One reason for the failure to completely ‘kill’ cancer cells has been attributed to the self-renewal properties of cancer stem cells, which contribute to both the initiation and relapse of tumors. PcG proteins such as BMI1, EZH2, and SUZ12 have been proven to promote the proliferative potential of stem cells in several cancer types. Interestingly, PcG proteins are activated upon exposure to known environmental carcinogens such as arsenic,²⁷⁹ cigarette smoke,²⁸⁰ and 7, 12-dimethylbenz [a] anthracene (DMBA).²⁸¹

In arsenic trioxide-induced transformed BALB/C 3T3 cells, BMI1 and SUZ12 are activated, leading to increased H3K27me3 levels, along with drastic decreases in the mRNA and protein expression levels of p16INK4a and p19ARF.²⁷⁹ Genetic knockdown of BMI1 or SUZ12 in BALB/C 3T3 cells results in the suppression of arsenic-induced malignant transformation, as well as decreased H3K27me3 levels.²⁷⁹ In murine DMBA-mediated skin tumors, down-regulation of tumor suppressor miR-203 (via promoter methylation) is observed, along with up-regulation of c-Myc and BMI1.²⁸¹ It is interesting to note that BMI1 itself is a target of miR-203.²⁸² However, in invasive cancer, this miRNA is often suppressed by DNA methylation *via* the pro-invasive molecule, ZEB1, leading to increased levels of BMI1.²⁴⁵ On the other hand, cigarette smoke enhances the initiation and progression of lung cancer *via* inhibition of the Wnt inhibitor, Dkk-1. The down-regulation

of Dkk-1 is accompanied by increased H3K27me3 and the recruitment of BMI1, EZH2, and SUZ12 within the Dkk-1 promoter.²⁸⁰ Thus, cigarette smoke utilizes the polycomb machinery to activate a signaling network involved in enhancing the malignant phenotype of lung cancer cells. These findings reveal the roles of PcG activation in mechanisms underlying carcinogenesis and cancer progression. Therefore, inhibition of PcG activation and its cooperative oncogenic partners might be an attractive and effective approach toward chemoprevention.

Several natural compounds that inhibit PcG activation in cancer cells and tissues have been identified. Epigallocatechin gallate (EGCG), a polyphenol antioxidant found in green tea, decreases the levels of BMI1 and EZH2 in squamous skin carcinoma cells.²⁸³ This is associated with global attenuation of H3K27me3, leading to down-regulation of cell cycle progression-related proteins (*e.g.*, cyclin D1 and cyclin E) and survival proteins (*e.g.*, Bcl-xL). Similarly, treatment with sulforaphane (SFN), an isothiocyanate found in cruciferous vegetables, leads to a concentration-dependent reduction in the expression of BMI1 and EZH2 in SCC-13 skin cancer cells, along with a reduction in H3K27me3 and G2/M phase cell cycle arrest. Further, that study demonstrates that the reduction in PcG protein levels is associated with their proteasomal degradation.²⁸⁴ In nasopharyngeal carcinoma cells, the antimalarial sesquiterpene, artemisinin, inhibits BMI1 at both mRNA and protein levels, arresting cells in the G1 phase and increasing the p16 levels.²⁸⁵ Wedelolactone, an essential active compound of *Eclipta prostrata*, binds to EED with a high affinity ($K_D = 2.82 \mu\text{M}$), disrupts the interaction of EZH2 and EED. In PRC2-dependent cancer cells, Wedelolactone inhibits the cell proliferation and induce apoptosis by modulating PRC2 targets and cancer-related genes expression.²⁸⁶ In bladder cancer, NSC745885, an emodin derivative, overcomes multiple-drug resistance, down-regulates EZH2 via proteasome-mediated degradation and suppresses tumor growth both *in vitro* and *in vivo*.²⁸⁷ However, studies have yet to explore the validity of PcG protein reduction as an effective cancer prevention approach in the clinical setting.

B. Cancer Therapy

The oncogenic potential of PcG proteins suggests that pharmacological or genetic targeting of the polycomb machinery would be an attractive approach for cancer therapy. In mammalian cells, chromatin surrounds the genetic material within an exclusive structure consisting of histones and associated multimers, such as the PRCs and miRNAs. These components influence the genetic expression patterns without changing the DNA base pairs, which is known as epigenetic programming. Interestingly, the stem cell chromatin, being in a more plastic and hyperdynamic state compared to lineage-committed and differentiated cells, contains both activating and repressive types of histone modifications.³² The current evidence indicates that the deregulation of chromatin programming in normal stem cells affects their self-renewal properties, leading to cancer development and progression. Below, we describe some of the current strategies being used to target PcG proteins for cancer therapy.

1. RNAi-mediated Inhibition of PRCs—Genetic disruption of PcG silencing complexes has been tested in several preclinical models with promising results. In multiple myeloma

cells, RNA interference (RNAi)-mediated BMI1 silencing sensitizes tumor cells to bortezomib and radiation, along with a reduction in the levels of the antiapoptotic protein, Bcl-2.²⁸⁸ A siRNA-mediated BMI1 knockdown also suppresses tumor growth and induces apoptosis *in vitro* and *in vivo* in laryngeal carcinoma²⁸⁹ and decreases the invasive potential of gastric carcinoma cells.²⁹⁰ ShRNA-mediated knockdown of BMI1 inhibits cell migration, induces cell apoptosis, and reduces CD44(+)CD133(+) sub-population in tongue cancer.²⁹¹ The down-regulation of BMI1 has been associated with increased chemosensitivity in various cancers.^{292,293} Similar findings have been reported for PRC2 components like SUZ12²⁹⁴ and EZH2.²⁹⁵⁻²⁹⁷ RNAi mediated SUZ12 depletion leads to cell numbers reduction and cell cycle arrest in gastric cancer. SUZ12 knockdown increases p27 protein level, and decreases p27 promoter methylation.²⁹⁴ EZH2 depletion mediated by RNAi induces apoptosis and cell cycle arrest by reducing multidrug resistance protein 1 (MDR1) expression in multidrug-resistant HCC Bel/FU cells. EZH2 knockdown also reverses the resistance of Bel/FU cells to chemotherapy.²⁹⁵ Same findings also have been observed in colon cancer,²⁹⁶ atypical teratoid/rhabdoid tumor,²⁹⁷ and medulloblastoma.²⁹⁸ Stable EZH2 knockdown induces apoptosis, cell cycle arrest and anaphase bridging, sensitizes topoisomerase II (TopoII) inhibitor in bcrhl-related gene 1 (BRG1) or epidermal growth factor receptor (EGFR) mutant non-small-cell lung cancer cells.²⁹⁹ Intriguingly, Ezh2 knockdown by doxycycline-inducible shRNAs decreases H3K27me3 levels, inhibits proliferation, induces G0/G1 phase arrest and plasticity in glioma-initiating cells, and significantly improved animal survival. Contrarily, prolonged Ezh2 depletion significantly enhances proliferation, induces DNA damage repair genes, activates partial pluripotency network, and attenuates chemotherapeutic drug treatment, implicating that precise dosing regimen is very important in the clinic.³⁰⁰

2. Gene Therapy: Adenoviral RYBP Delivery—We and Novak *et al.*, have shown that adenovirus-mediated RYBP gene therapy efficiently induces apoptosis both *in vitro* (HCC¹⁶⁰, lung cancer and sarcoma cell lines²⁷⁸) and *in vivo* (mouse HCC tumor xenografts).¹⁶⁰ In HCC, intratumoral RYBP delivery potentiates the action of cisplatin, inducing apoptosis *via* the down-regulation of MDM2 and up-regulation of Bax. Interestingly, the apoptotic effects of RYBP do not depend on the p53 status of the cells.¹⁶⁰

3. Pharmacological Inhibition of PRCs

a. Specific PRC1 Inhibitors (Small Molecules and peptides): PTC-209, a small-molecule BMI-1 inhibitor, can inhibit colorectal cancer cell growth and colorectal cancer-initiating cells (CIC) self-renew. PTC-209 inhibits tumor growth of colorectal cancer xenografts and reduces colorectal CIC frequency, suggesting BMI-1-related self-renewal is a viable therapeutic strategy.³⁰¹ PTC-209 also shows more critical cytotoxic effects in acute myeloid leukemia (AML) than in ALL cells. Treatment with PTC-209 causes BAX conformational change, activates caspase-3 and DNA fragmentation, decreases mitochondrial membrane potential (MMP), and externalizes phosphatidylserine (PS).³⁰² PTC596 (from PTC Therapeutics), an orally bioavailable selective SMI1 inhibitor, modifies BMI1 post-translation, degrades BMI1 protein and reduces PRC1 activity in glioblastoma and fibrosarcoma and leukemia. Oral administration of PTC596 inhibits the tumor growth, depletes the tumor stem cell fraction and significantly extends the lifespan.³⁰³

Mechanistically, PTC596 inhibits anaphase-promoting complex/cyclosome (APC)/CCDC20 activity and increases the CDK1 and CDK2 which mediate BMI1 hyperphosphorylation.³⁰⁴ The candidate now has entered into clinical trials ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02404480) Identifier: NCT02404480) for advanced solid tumor treatment.

Recently, Tang *et al.* have firstly created chromodomain antagonists targeting CBX7 by a peptide-driven approach. A series of compounds that have a ~200nM potency and selectivity with CBX7 binding are identified.³⁰⁵

b. Specific PRC2 Inhibitors (Small Molecules and Peptides): Another promising avenue of treatment is the pharmacological inhibition of PRCs. It has been demonstrated that exposure of cancer cells to the S-adenosylhomocysteine hydrolase (SAH) inhibitor 3-Deazaneplanocin A (DZNep) and its analogs disrupts PRC2 and depletes the EZH2 levels, causing the reactivation of silenced pro-apoptotic genes,³⁰⁶⁻³⁰⁹ induction of autophagy,³¹⁰ upregulation of senescence promoting genes (*e.g.*, p16³¹¹), and the upregulation of tumor suppressor miRNAs (*e.g.*, miR-302 and miR-4448³¹²). In addition, it has been reported that DZNep works synergistically with HDAC inhibitors and DNMT inhibitors to activate PRC2 silenced genes.³¹³ DZNep has been shown to cause selective cell death in the different cancer cell types tested, without affecting normal cells.³⁰⁶ Moreover, it appears that the responsiveness of cancer cells to DZNep is determined by the p53 status of the cells.^{309,314} In p53 wild-type thyroid cancer cells, DZNep causes p53 protein accumulation through the upregulation of USP10 expression, resulting in activation of the p53 pathway and inhibition of cell growth. Conversely, p53 mutant cells are resistant to DZNep. Restoration of wild-type p53 by the mutant p53 reactivator PRIMA-1 restores the sensitivity of p53 mutant cells to DZNep both *in vitro* and *in vivo*.³¹⁴

EZH2 mediates its catalytic methyltransferase activity (responsible for gene silencing) through its C-terminal SET domain. This domain contains two essential binding pockets: one for the SAM methyl donor and another for the K27 substrate.¹⁴¹ Subsequent high-throughput screening studies have been carried out to identify novel EZH2 inhibitors that inhibit the methyltransferase activity of EZH2 and possess Ki values in the low nanomolar range. EPZ005687 (from Epizyme Inc.) shows selective cytotoxicity against lymphoma cell lines and inhibits H3K27me3 by the EZH2 mutants Y641 and A677.³¹⁵ EPZ-6438 (from Epizyme Inc.) selectively inhibits H3K27Me3 in both EZH2 wild-type and mutant non-Hodgkin lymphoma (NHL) cells and completely induces tumor regression after oral administration.³¹⁶ EPZ-6438 recently is undergoing clinical trials as E7438 for patients with B cell lymphomas or with advanced solid tumors ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01897571) Identifier: NCT01897571). EPZ011989 (from Epizyme Inc.), a modification of the pyran substituent in EPZ-6438, specifically inhibits both mutant and wild-type EZH2 with a very low inhibition constant (Ki < 3 nM). It significantly inhibits human B cell lymphoma cell growth both *in vitro* and *in vivo*. EI1 (from Novartis) is equally active against both the wild-type and Y641 mutant forms of EZH2, and induces apoptosis in B-cell lymphomas.³¹⁷ GSK126 (from GlaxoSmithKline) effectively inhibits cell proliferation and decreases global H3K27me3 levels in EZH2 mutant diffuse large B-cell lymphoma (DLBCL) cell lines and mouse xenografts.³¹⁸ Ovarian clear cell carcinomas (OCCC) with AT-rich interactive domain 1A (ARID1A) mutation have no effective therapy currently. GSK126 can selectively inhibit cell

proliferation and induce apoptosis in *ARID1A*-mutated OCCC cells. GSK126 treatment causes tumor regression and decreases ARID1A-mutated dissemination *in vivo* and PI3K-interacting protein 1 (PIK3IP1) contributes toward this synthetic lethality, suggesting EZH2 methyltransferase activity inhibition is a new therapeutic strategy for *ARID1A*-mutated cancers treatment.³¹⁹ UNC1999, an orally bioavailable analogue of GSK126, exhibits high *in vitro* potency against wild-type and mutant EZH2, alone or in combination with an EGFR inhibitor in colon cancer model.³²⁰ UNC1999 also selectively suppresses global H3K27me3/2, increases H3K27ac, and inhibits cell growth of mixed lineage leukemia (MLL)-rearranged leukemia both *in vitro* and *in vivo*.³²¹ GSK2816126 (from GlaxoSmithKline), a SAM competitive inhibitor of EZH2 has advanced into clinical trials (clinicaltrials.gov identifier: NCT02082977). CPI-169, a potent and selective EZH2 inhibitor, shows excellent efficacy in EZH2 mutant lymphoma xenograft models. Mechanistically, selectively affects H3K27me3, but not H3K27me1 at pharmacologically relevant doses, suggesting efficacy of CPI-169 depends on comprehensive EZH2 inhibition, but not require EZH1 inhibition.³²²

Recent exome sequencing studies in glioblastoma multiforme (GBM) and pediatric diffuse intrinsic pontine gliomas (DIPG) have identified a novel missense mutation (K27M) in the genes encoding histone H3.3 (*H3F3A*) and H3.1 (*HIST1H3B*) through a yet poorly understood gain-of-function mechanism.³²³⁻³²⁵ In GBM, 31% recurrent mutations in H3F3A are identified.³²⁶ 78% of DIPGs and 22% of non-brainstem pediatric glioblastomas also contain a mutation in H3F3A or HIST1H3B.³²⁶ The H3K27M mutation is shown to inhibit the global PRC2 enzymatic activity through interact with EZH2 both *in vitro* and directly in DIPG tumor. H3.1 or H3.3 transgenes possessing the K27M mutation both lead to a striking reduction of H3K27me3 on the non-mutated H3, both within the same nucleosome and on nearby nucleosomes. In addition, in H3.3K27M patient cells, a remarkable gain of H3K27me3 and EZH2 also are observed at hundreds of chromatin loci and alter the various cancer pathways related genes expression.³²⁷ Allis and colleagues have recently filed a US patent application for isolated peptides derived from H3K27M that function as potent PRC2 inhibitors (US20140107039). However, no studies of such inhibitors have been carried out on preclinical animal models.

Kim and colleagues develop a stabilized α -helix (SAH) that mimicked the EZH2 alpha-helical EED-binding domain. This stapled peptide, SAH-EZH2, disrupts EED and EZH2/EZH1 interaction, and selectively inhibits H3K27 methylation and reduces EZH2 levels. In MLL-AF9 murine leukemia cells, SAH-EZH2 inhibits cell proliferation, induces cell cycle arrest and monocyte-macrophage differentiation.³²⁸

c. Known Anticancer Drugs That Inhibit PRC: Several commonly used anticancer drugs that reverse the abnormal epigenetic changes during oncogenesis also reduce the expression of PcG proteins. Currently, these can be divided into two major classes: DNA methyltransferase (DNMT) inhibitors and HDAC inhibitors, which epigenetically reprogram cancer cells toward a more normal phenotype. A number of these epigenetic modifiers are currently in clinical trials.^{329,330} *In vitro* studies involving a combined regimen where DNMT inhibitors like 5-azacytidine are administered first, followed by HDAC inhibitors, have shown promising results.¹⁸⁸ Of note, HDAC inhibitors induce cancer cell apoptosis,

and this is enhanced by the combination therapies.³³¹ Broad-spectrum HDAC inhibitors, such as sodium butyrate (NaB) and valproic acid (VPA), also lead to significant decreases in the BMI1 and EZH2 protein levels, which are accompanied by decreases in H2AK119Ub.³³²⁻³³⁴ These events are associated with the re-expression of growth inhibitory proteins and tumor suppressor genes, resulting in senescence or apoptosis. ACY-957, a HDAC1,2 selective inhibitor, increases global H3K27ac and induces DNA damage and impairs DNA repair in vitro and sensitizes the EZH2 gain-of-function mutant (EZH2GOF) DLBCL cells to doxorubicin.³³⁵ Astemizole, an old anti-histamine drug, has been suggested as a new promising anticancer agent since it targets important proteins involved in cancer progression, including ether à-go-go-1 (Eag1) potassium channels. Recently, Astemizole is identified as an EZH2-EED interaction small molecule inhibitor. Astemizole inhibits the PRC2 complex activity and decreases H3K27me3 levels, resulting in anti-proliferation activity in Lymphomas.³³⁶ However, a potential complication of such an approach may be the varied widespread effects on normal cell biology, since PRC target genes are ubiquitously expressed in all cell types.

7. Conclusions and Future Directions

PcG proteins form the bridge between epigenetics and cancer stem cell biology, and are therefore garnering tremendous interest in cancer research. The PcG proteins are involved in various steps of cancer progression, and have been demonstrated to affect both the initial malignant transformation and distant metastasis. Although the roles of several individual PcG proteins have been discussed in this review, future studies should systematically investigate the role of each PcG protein in different cancer types. As discussed in previous sections, PcG proteins can have both tumor suppressor and oncogenic roles, in addition to possessing multiple layers of regulation by both genetic and epigenetic regulators. We believe that one of the most exciting findings in recent polycomb research is the identification of the transcriptional activation roles of polycomb proteins, which have generally been regarded as global gene silencers. These novel interactions and functions of the PcG family members offer new insights into their roles as key players of cancer development and progression, and these roles must be investigated in greater detail.

Due to the large differences in their effects on progression in different cancer types, the impact of molecules aiming to inhibit the oncogenic PcG functions should be investigated by well-designed pharmacogenomic studies. The results already obtained with the EZH2 inhibitors indicate that the pharmacological targeting of PRCs is an attractive therapeutic strategy, despite some concerns regarding the specificity of these drugs and their pharmacokinetic profiles. In the future, more specific inhibitors targeting one or more PcG members can be developed. Future studies should evaluate the synergy between pharmacological PcG inhibitors and the currently employed chemotherapeutic and epigenetic modifiers. Since EZH2 modulates genes involved in the EMT and invasion, PRC2 targeting may impair tumor metastasis and angiogenesis. Thus, the specific activity of DZNep and other EZH2 inhibitors on these aspects of tumor progression should be evaluated. Such studies will require a multi-pronged approach, involving tumor xenografts, the use of *in vivo* models that represent the epigenetic landscape of human cancers, and pharmacogenetics to highlight the clinical value of PcG inhibitors. Finally, genetically

engineered mice overexpressing specific PcGs in tumor tissues may be used to represent a subset of patients who might derive particular benefit from treatment involving the inhibition of aberrant PcG expression. Thus, drugs targeting PcGs should be introduced into the clinical setting after systematic testing in multiple *in vivo* models, and after the identification of appropriate candidate biomarkers of tumor response. We believe such a systematic approach may help unlock the full potential of this complicated but indispensable network of molecules that is at the very heart of our existence.

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Biographies

Wei Wang is an Assistant Professor in the Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center (TTUHSC), Amarillo, TX, USA. She obtained her M.D. in Clinical Medicine from Changzhi Medical College, Changzhi, China and her Ph.D. in Molecular Pharmacology from Fudan University, Shanghai, China. Prior to joining the faculty at TTUHSC, she completed her post-doctoral training and also worked as a Research Associate at the University of Alabama School of Medicine, Birmingham, AL, USA. Dr. Wang's research has been continuously focused on targeted molecular cancer therapy, with particular interests including cancer cell signaling pathways, experimental therapeutics and drug development.

Jiang-Jiang Qin is a Research Associate in the Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center (TTUHSC), Amarillo, TX, USA. He obtained his B.S. in Pharmaceutical Sciences and his Ph.D. in Biomedical Engineering (Genetic Engineering and Natural Medicine) from Shanghai Jiao Tong University, Shanghai, China. His research interests include signaling pathways in carcinogenesis, cancer progression, prevention, and treatment.

Sukesh Voruganti is a Ph.D. candidate in the Graduate School of Biomedical Sciences, the program of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, Amarillo, TX, USA. He completed his bachelor's degree at Jawaharlal Nehru Technological University, Hyderabad, India. His research focuses on novel small molecular anticancer drugs, gene therapy, preclinical pharmacology and toxicology, and drug delivery.

Subhasree Nag has recently obtained her Ph.D. in Pharmaceutical Sciences from Texas Tech University Health Sciences Center, Amarillo, TX, USA. She also completed her master's degree at the Institute of Chemical Technology, Mumbai, India. Her research focuses on the preclinical pharmacology of anticancer agents and pharmaceutical analysis.

Jianwei Zhou is a Professor in the Department of Molecular Cell Biology and Toxicology, School of Public Health, and Dean of the School of Foreign Graduates and Department of Foreign Affairs, Nanjing Medical University, Nanjing, China. He obtained his M.D. in Public Health and his Ph.D. in Occupational and Environmental Health at Nanjing Medical College, Nanjing, China. He was an exchange scholar at the University of California, Davis, CA, USA between 1996 and 1998. His research interests include molecular and cellular biology, cancer biology, environmental toxicology, and experimental therapy.

Ruiwen Zhang is a Professor in the Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center (TTUHSC), Amarillo, TX, USA. He obtained his M.D. and Ph.D. in Toxicology from Shanghai Medical University (now Fudan University Shanghai Medical College), Shanghai, China. He completed his

Postdoctoral/Clinical Pharmacology Fellowship at the University of Alabama School of Medicine, Birmingham, AL. USA, where he joined the faculty in the Division of Clinical Pharmacology and the Department of Pharmacology and Toxicology and was then became Professor and Director of the Cancer Pharmacology Laboratory. He is certified by the American Board of Toxicology (D.A.B.T.) and was on its Board of Directors from 2009 until 2013. Dr. Zhang was elected as a Fellow of the American Association for the Advancement of Science (AAAS) in 2009. His major research interests include translational medicine, cancer biology, gene silencing, drug discovery and experimental therapy, clinical pharmacology and toxicology, focusing on targeting oncogenes and tumor suppressor genes.

Figure 1. Epigenetic regulation of PcG proteins during differentiation and carcinogenesis

In embryonic stem cells (undifferentiated pluripotent cells), the genes crucial for development are marked by a specific 'bivalent domain' structure, composing of the active H3K4 methylation (H3K4me) and the repressive H3K27 trimethylation (H3K27me3) marks, which maintain the epigenomic plasticity. During differentiation, these bivalent domains transform into a more rigid 'monovalent domain' structures that is either active (with H3K4 methylation) or repressive (H2AK119 ubiquitination/H3K27 trimethylation), depending upon which mark is maintained. Based on the cell type, particular subsets of genes are expressed and silenced, ultimately leading to the generation of morphologically and functionally different cells. During cancer development and progression, both embryonic stem cells and differentiated cells undergo aberrant reprogramming of polycomb proteins that result in gene silencing through the formation of a compact chromatin structure. Silencing can occur through PRC reprogramming-silencing of active genes by the polycomb group, silencing through *de novo* hypermethylation, accompanied by H3K9 methylation (H3K9me).

Figure 2. Mechanisms of transcriptional silencing by PRCs

PRC1 complexes catalyze histone H2A lysine 119 (H2AK119Ub1) monoubiquitination. The catalytic subunits of PRC1 consist of RING1/2 and one of six PCGF protein homologues, PCGF1-6. PRC1 is typically involved in both the canonical and non-canonical pathways. Canonical PRC1 complexes contain RING1/2 ubiquitin ligase, PCGF2/4, the CBX subunit, and the PHC proteins. The association of additional subunits with non-canonical PRC1 occurs in a PCGF-dependent manner. Thus, PRC1 variant complexes include the additional subunits BCOR and KDM2B. PRC2 complexes consist of the catalytic EZH2 subunit and the core subunits EED, SUZ12, and RbAp48. The Jumonji C-containing protein, JARID2, binds to most PcG target genes, and is itself required for the binding of PcGs to their target genes in ES cells. However, JARID2 inhibition has minimal effects on the global H3K27me3 levels.

Figure 3. PcG protein recruitment to target genes

(A) A high binding ratio between the homologous proteins Pho (P) and PhoI (PI) is seen at polycomb response elements (PREs), which is essential for targeting and anchoring PRC2 and PRC1 to PREs. PcG protein complex recruitment to PREs occurs in conjunction with the previously identified PcG protein recruiters such as dorsal switch protein 1 (Dsp1), Pho, and Phol. In addition, non-coding RNAs (ncRNAs) help to recruit PcG protein complexes. The recruitment of PcG protein complexes to PREs might be mediated by DNA-binding proteins (indicated by X). (B) Transcription factors (TF), which act as co-activators for the transcription of target genes, might block the recruitment of PcG protein complexes at non-PcG binding sites.

Figure 4. Polycomb-independent transcriptional activation by EZH2

(A) In ER-negative breast cancer cells, EZH2 activates NF κ B target genes through the formation of a ternary complex with the NF κ B components, RelA and RelB, via a process that does not require other PRC2 subunits. (B) In ER-positive breast cancer cells, EZH2 physically interacts directly with ER- α and Wnt signaling components, activating their downstream targets, like c-myc and cyclin D1, via RNA polymerase II transcription. (C) In castration-resistant prostate cancer (CRPC), phosphorylation of EZH2 at Ser21, mediated by the PI3K-Akt pathway, alters its function from a polycomb repressor to a transcriptional co-activator of the AR.

Figure 5. Non-polycomb functions of BMI1

(A) The PRC1 subunit, BMI1, acts as an activator of the WNT pathway by repressing the Dickkopf (DKK) family of WNT inhibitors. The Wnt/β-Catenin pathway regulates stem cell pluripotency and cell fate decisions during development. In the presence of the Wnt ligand, the co-receptor LRP5/6 forms a complex with Wnt-bound frizzled, activating disheveled (Dvl) by sequential phosphorylation, poly-ubiquitination, and polymerization, which displaces GSK-3β from APC/axin, causing the nuclear translocation of β-catenin and the subsequent recruitment of TCF DNA-binding factors as co-activators for transcription. BMI1 represses the expression of the DKK proteins. DKK1 repression leads to the upregulation of the WNT target, c-Myc, which leads to further transcriptional autoactivation of BMI1. (B) The binding of TNFα to the TNF receptor (TNFR), causes the recruitment of TNFR1-associated death domain protein (TRADD), receptor-interacting protein (RIP) and TNF receptor-associated factor 2 (TRAF2) to the cell membrane. Then, the IκB kinase (IKK) complex, composed of IKKα, IKKβ, and IKKγ/NFκB essential modulator (NEMO), is recruited to the TNFR1 signaling complex, which leads to IKKβ phosphorylation and activation. This results in the nuclear translocation of NFκB and its subsequent transactivation. BMI1 stimulates IKKβ phosphorylation and the nuclear translocation of NFκB, which stimulates c-Myc. This, in turn, activates BMI1, leading to a positive feedback loop.

Figures 6. Role of RYBP in apoptosis and the PcG-MDM2-p53 pathway

RYBP enhances Fas-induced apoptosis. When the Fas receptor is bound by a ligand, the adaptor molecule Fas-associated protein with death domain (FADD), interacts with procaspase-8 and procaspase-10 to form the death-inducing signaling complex (DISC). RYBP interacts with FADD and caspases-8 and -10, enhancing the formation of the DISC and promoting Fas-mediated apoptosis. RYBP also binds to HIPPI and promotes neuronal apoptosis. RYBP forms a ternary complex with p53 and MDM2, preventing p53 ubiquitination and degradation. On the other hand, ring finger protein 2 (RNF2), an E3 ubiquitin ligase, promotes p53 ubiquitination but stabilizes MDM2, inhibiting its autoubiquitination.

Figure 7. Schematic representation of the aberrant polycomb signaling in cancer

The functions of many proteins from polycomb complexes are altered by aberrant expression, missense mutations, or chromosomal translocations in human cancers, likely leading to changes in transcriptional programs and cell states. The overexpression or aberrant expression of polycomb proteins leads to uncontrolled cell growth, invasion, resistance to cell death, and defective stem cell renewal patterns. During development, polycomb proteins mediate appropriate body planning and differentiation (A: anterior; P: posterior), which is disrupted in cancerous cells and defective stem cells. Non-coding RNAs (ncRNA) mediate polycomb gene silencing by helping recruit PcG protein complexes. In cancer cells, tumor suppressor miRNAs are downregulated. These miRNAs typically target the oncogenic polycomb proteins, leading to their degradation.

Figure 8. PcG proteins and miRNAs

(A) The expression of Ezh2, SUZ12, and Bmi1 is regulated at the post-transcriptional level by miRNAs, targeting their 3'UTR, and leading to transcript degradation. The miRNA complex is formed by TRBP (the human immunodeficiency virus transactivating response RNA-binding protein), argonaute 2 (Ago2), and dicer, which facilitates the recognition of PcG protein transcripts or marks them for cleavage. (B) Regulation of miRNA expression by polycomb proteins (e.g., the miR31-PRC2-NFκB axis). PRC2 overexpression leads to H3K27Me3 deposition on MiR-31 and its subsequent silencing. This leads to the overexpression of NIK and activation of NFκB-mediated survival and inflammatory pathways.

Figure 9. Targeting PcG proteins for cancer therapy

Aberrant gene silencing in cancer involves transcriptional repressive complexes (such as PRC1/PRC2) in the gene promoter region, and interactions between DNA methylation machinery and chromatin modifiers (such as histone deacetylase, HDAC). Pharmacological inhibition of individual components of the transcriptionally repressive chromatin with DNMT inhibitors, HDAC inhibitors, or PRC inhibitors that inhibit the H3K27Me mark either alone or in combination, may result in DNA demethylation and complex disintegration, leading to the reactivation of critical tumor suppressor genes, including those associated with lineage commitment, immunomodulation, major cell signaling pathways, programmed cell death, and other processes. HAT: histone acetylase. Pol II: RNA polymerase II.

Table I
Representative members of the PRC complexes and their functions

Drosophila	Mouse/human homolog	Polycomb functions	Biological functions	Phenotype of knockout mice	References
Polycomb repressive complex 1 (PRC1)					
Pc	CBX2	Binding to H3K27me3	Involved in sexual development, acting as an activator of NR5A1 expression	Viable mice with gonadal, adrenal, and splenic defects and homeotic transformation	[63-65]
	CBX4	SUMO E3 ligase, binding to RNA, H3K9me3, and H3K27me3	Involved in the sumoylation of HNRNPK, a p53 transcriptional co-activator, resulting in p21 expression	Severe hypoplasia of the fetal thymus as a result of reduced thymocyte proliferation	[66-68]
	CBX7	Binding to H3K27me3, H2AK119 ubiquitination	Regulator of cellular lifespan by maintaining the repression of CDKN2A	Spontaneous solid tumor development	[69-71]
PSC	BMI1 (PCGF4, RNF51)	Chromatin compaction and H2AK119 ubiquitination	Maintains adult stem cells in organs	Viable mice with posterior homeotic transformation, severe immunodeficiency, and neurological abnormalities	[72-75]
	MBL1 (PCGF6, RING6, RNF134)	H3K4 demethylation		Not reported	[73,76,77]
	NSPC (PCGF1, RNF68)	H3K36 demethylation	Regulation of the differentiation and self-renewal of hematopoietic cells; promotes EMT via E-cadherin regulation	Not yet reported	[78,79]
See (RING)	RING1A/1 (RNF1)	H2AK119 ubiquitination, RNA Pol II block		Viable mice with skeletal patterning abnormalities	[80,81]
	RING1B/2 (RNF2)	H2AK119 ubiquitination, RNA Pol II block, chromatin compaction		Developmental arrest in early gastrulation	
dRYBP	RYBP	H2AK119 ubiquitination, PRC1 recruitment	Involved in Fas- mediated apoptosis and p53 stabilization	Post-implantation embryonic lethality, eye and CNS defects	[82-86]
	YAF2	Bridging of the interaction between YY1 and the PRC1 complex	Regulates cell survival during embryogenesis; inhibits transactivation and transformation	Not yet reported	[87-90]
Polycomb repressive complex 2 (PRC2)					
E(Z)	EZH1	H3K27 trimethylation, chromatin compaction	Hematopoietic stem cell maintenance		[57,91]
	EZH2	H3K27 trimethylation, H3K27me3 recognition, binding to RNA, chromatin-loop formation, protein methylation	Failure to undergo mesoderm differentiation	Embryonic lethality post-implantation	[56, 92,93]
Su(Z)12	SUZ12	PRC2 assembly, RNA binding	Lethal at post-implantation stage, developmental arrest		[94,95]

Drosophila	Mouse/human homolog	Polycomb functions	Biological functions	Phenotype of knockout mice	References
ESC	EED	H3K27me3 recognition, RNA binding	Provides a recruiting platform for DNA methyltransferases, may regulate integrin function	Disrupted axial patterning, defects in gastrulation	[96-98]
	JARID2	Histone demethylation, PRC2 recruitment		Organ abnormalities	[99,100]
	RbAp46/48	RNA binding	Inhibition of transcriptional transactivation mediated by BRCA1	Conditional knockout mice show defective peripheral lymphocytes	[96,101]
PCL	PCL1-3	PRC2 recruitment, stimulation of PRC2 activity	Stem cell renewal		[102-104]
PHO repressive complex 1 (PHORC)					
PHO	YY1	PRC1 and PRC2 recruitment	Regulation of mammalian spermatogenesis	Embryonic lethal perimplantation	[105]

Table II
Deregulation of PcG genes in human cancers

Subunit	Expression/mutation	Cancer type	Clinical outcome	References
PRC1 components				
CBX7	Protein overexpression	Follicular lymphoma	Increased risk and poor prognosis	[170]
	Loss of both alleles	Pancreatic cancer	Increased risk and poor prognosis	[171]
PHC1	Loss of heterozygosity	Acute lymphoblastic leukemia	Malignant transformation	[172]
PHC3	Loss of heterozygosity	Osteosarcomas	Malignant transformation	[173]
	Missense mutation	Osteosarcomas	Increased risk	[174]
BMI1	Protein overexpression, Gene amplification	Hematological malignancies (acute myeloid leukemia, lymphoma), Solid tumors (lung cancer, neuroblastoma, medulloblastoma,)	Increased risk, poor prognosis; increased levels correlate with advanced forms of the disease	[175-178]
PCGF2/MEL18	Loss of both alleles	Prostate and gynecological tumors	Poor prognosis	[179,180]
RING1	Loss of both alleles	Clear-cell renal-cell carcinomas, testicular germ-cell tumors	Poor prognosis	[180]
RING2	Protein overexpression	Solid tumors (liver, uterine, cervical, lung, breast, prostate, bladder, oral cavity, and colon cancers)	Increased invasive capabilities	[180]
ASXL1	Frameshift, truncation	Hematological malignancies	Increased risk	[181-184]
	Missense mutation	Hematological malignancies	Poor prognosis	[181-184]
ASXL2	Chromosomal translocation; fusion gene partner: EPC1	Acute lymphoblastic leukemias	Increased risk and poor prognosis	[185]
PRC2 Components				
EZH2	Protein overexpression Gene amplification	Hematological malignancies (B-cell non-Hodgkin lymphomas, mantle cell lymphoma); Solid tumors (breast, prostate, bladder, pancreatic, oral cavity, and colon cancer; glioblastoma)	Endocrine therapy resistance and a shorter progression-free survival in breast cancer Mediates progression to hormone-refractory form of prostate cancer	[153,154, 186-188]
	Missense mutation	Hematological malignancies	Poor prognosis and increased risk	[189]
SUZ12	Protein overexpression Gene amplification	Hematological malignancies (B-cell non-Hodgkin lymphomas, mantle cell lymphoma); Solid tumors (liver, breast, prostate, bladder, oral cavity, and colon cancer)	Poor prognosis and shorter overall survival Increased risk	[190-192]
	SUZ12 mutation	Malignant peripheral nerve sheath tumors (MPNST)	Malignant transformation	[193]
	Chromosomal translocation; fusion gene partner: JAZF1	Sarcoma	Increased risk	[194]
PCL3/PHF19	Protein overexpression	Liver, uterine, cervical, lung, breast, prostate, bladder, oral cavity, and colon cancers	Increased risk and poor prognosis; increased CSC population	[195]

Subunit	Expression/mutation	Cancer type	Clinical outcome	References
EPC1	Truncation mutation	Acute lymphoblastic leukemias	Poor prognosis and increased risk	[185]
PCL1/PHF1	Chromosomal translocation; fusion gene partner: JAZF1, EPC1	Sarcoma	Increased risk	[196]

Table III

PcG proteins in cancer development and progression

Subunit	Target gene(s)	Interactive partner(s)	Molecular mechanisms	Biological effects	References
Cell cycle/senesence					
PRC1 subunits (BMI1, PCGF1, PCGF2/MEL18, CBX2, CBX7, CBX8, and RING2B); PRC2 subunits (EED, SUZ12, and EZH2)	p16 ^{Ink4a} /p19 ^{Arf} locus	pRB	Transcriptional repression of the p16/p19 locus via direct binding to the p16 ^{Ink4a} /p19 ^{Arf} promoter and activation of the cyclin D-CDK 4/6 complex	Increased proliferation due to cell cycle progression in the G1 phase	[209]
PRC2 subunit EZH2	p15 ^{Ink4b}	pRB	Transcriptional repression of the p15 locus via binding and trimethylation of H3K27 in the p15 ^{Ink4b} promoter; subsequent activation of cyclin D-CDK 4/6 complex	Increased proliferation due to cell cycle progression in the G1 phase	[210,211]
PRC2 subunit EZH2	Cyclin A	pRB2/p130	Disrupts recruitment of HDAC1 to cyclin A promoter by pRB2/p130	Cyclin A activation and cell cycle progression in the G1 phase	[212]
PRC1 subunits (BMI1, RING1B, SCMH1, and PHC1)	Geminin		Ubiquitination and proteasomal degradation of geminin leading to the release of suspended DNA replication forks	Cell cycle progression in the G1 phase	[213]
PRC1 subunit BMI1	CDC6		Physical interaction with CDC6 and transcriptional repression of the INK4c/ARF locus	Cell cycle progression in the G1 phase	[214]
PRC1 subunits CBX4 and RING1	Cyclin A	pRB, E2F	Direct binding to cyclin A promoter and repressor of cyclin A and cdk1	G2/M arrest	[211]
PRC1 subunit BMI1	p21		Transcriptional inhibition of p21 via direct binding to the p21 promoter	Increased proliferation due to cell cycle progression in the G1 phase	[215]
PRC2 subunit EZH2	p57		Transcriptional inhibition of p57 via binding and trimethylation of H3K27 on p57 promoter	Increased proliferation due to cell cycle progression in the G1 phase	[216]
DNA damage repair					
PRC subunit Pc2	HIPK2		Pc2 sumoylates HIPK2, leading to transcriptional silencing of its gene targets	Not precisely defined	[217,218]
EMT					
PRC2 subunit EED	E-cadherin	TGFβ	Promotion of TGF-β-induced transcriptional repression of E-cadherin	Increased transition into mesenchymal state	[219]
PRC subunits EZH2/BMI1	E-cadherin	SNAIL	Transcriptional repression of E-cadherin via recruitment of PRC2 to the E-cadherin promoter by SNAIL	Increased transition into mesenchymal state	[134]
Migration and invasion					

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Subunit	Target gene(s)	Interactive partner(s)	Molecular mechanisms	Biological effects	References
PRC2 subunit EZH2	Integrin $\alpha 2$	Cofilin	Repression of integrin $\alpha 2$ by EZH2 decreases cofilin phosphorylation, increasing cell migration	Increased migration	[220]